General and Comparative Endocrinology

Title Changes in Plasma Progesterone, Estrogen and Testosterone Concentrations throughout the Reproductive Cycle in Female Viviparous Blue-tongued Skinks, Tiliqua nigrolutea, (Scincidae), in Tasmania.

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Short title Steroids in female T. nigrolutea

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Abstract

Changes in mean plasma concentrations of progesterone (P4), estrogen (E) and testosterone (T) in a captive population of female viviparous skinks, Tiliqua nigrolutea were examined. Reproductively active and quiescent individuals were present in the population concurrently, allowing a comparison between these two conditions. Mean plasma progesterone concentrations were basal (1 – 2 ng ml^{-1}) until the start of gestation and peaked in the second trimester (12.7 ± 1.27 ng ml^{-1}), before falling significantly prior to parturition. An increase in mean plasma estrogen concentrations occurred coincident with the vitellogenic period; mean plasma estrogen concentration peaked at 715.1 ± 106.68 pg ml^{-1} shortly before ovulation. Mean plasma testosterone concentrations in reproductively active females peaked in the periovulatory period (6.3 ± 0.63 ng ml^{-1}) and had returned to basal concentrations (< 1 ng ml^{-1}) two weeks later. Changes in mean plasma steroid concentrations were correlated with reproductive condition, and are discussed in terms of potential in vivo steroid interactions and the multihormone control of reproduction.

Key Words: estrogen, progesterone, multihormone control, reproduction, skink, steroid, testosterone, Tiliqua nigrolutea

In females of seasonally cycling squamate reptiles plasma concentrations of progesterone (P4) and 17β-estradiol (E2) vary throughout the year, suggesting roles for each steroid in the regulation of various stages of the annual reproductive cycle. Traditionally, a third primary gonadal steroid, testosterone (T), is often overlooked, but T is also likely to have important functions in the regulation of reptilian reproduction (Owens, 1997; Staub and De Beer, 1997). There are, however, few comprehensive published descriptions of the annual profiles of all three primary steroids in the plasma of female viviparous reptiles.

Progesterone plays two important roles in the maintenance of gestation in viviparous reptiles (Highfill and Mead, 1975a; Naulleau and Fleury, 1990). One of these may be an indirect role through the antigonadal properties ascribed to P4 (Callard et al., 1972a): elevated P4 is thought to inhibit E2-induced follicular growth during pregnancy (Callard et al., 1972b; Guillette et al., 1981; Ho, 1987), ensuring that vitellogenesis and gestation are mutually exclusive (Callard et al., 1992). This is particularly important in viviparous temperate zone reptiles, which usually produce only a single clutch each year (Dunham et al., 1988). Progesterone also has a role in the maintenance of gestation in viviparous reptiles (Highfill and Mead, 1975a; Naulleau and Fleury, 1990), acting to inhibit oviductal contractility (Guillette et al., 1981; Guillette and Jones, 1985) and delay parturition (Callard et al., 1972a, b; Highfill and Mead, 1975b).

In most squamates, elevated plasma E2 concentrations are associated with the vitellogenic phase of the reproductive cycle (Bona-Gallo et al., 1980; Joss, 1985; Moore and Crews, 1986; Van Wyk, 1994; Jones et al., 1997). 17β-Estradiol is almost exclusively the hormone responsible for the stimulation of hepatic vitellogenesis in reptiles (Callard et al., 1972b; Callard and Ho, 1987;

Ho, 1987; Kime, 1987; Diaz et al., 1994). It may also have a role in the uptake of vitellogeni n by the follicle, as it is known to induce cellular endocytosis (Callard and Ho, 1987). 17 β-Estradiol is synthesised by the growing follicles (McNicol and Crews, 1979; Etches and Petitte, 1990) and plasma concentrations fall rapidly at or around the time of ovulation (Callard et al., 1978; Bona-Gallo et al., 1980; Yokoyama and Yoshida, 1994). As with circulating P4 concentrations, annual patterns of circulating E2 concentrations vary among viviparous squamates.

Little is known about the functional signifi cance of T in reproduction in female reptiles beyond its importance as a precursor in the synthesis of estrogens (Staub and De Beer, 1997). Published studies focus on oviparous species, in which a cyclic pattern of plasma T concentrations is apparent (Arslan et al., 1978; Callard et al., 1978; Bona-Gallo et al., 1980; Callard and Kleis, 1987; Whittier et al., 1987; Cree et al., 1992; Saint Girons et al., 1993; Rostal et al., 1998). The T in the plasma is likely to be of both ovarian and adrenal origin (Stau b and De Beer, 1997; Wade, 1997). Testosterone may be involved in the stimulation of vitellogenesis in the snakes Thamnophis sirtalis parietalis (Whittier et al., 1987) and Naja naja (Bona-Gallo et al., 1980), and in the hypertrophy of the oviduct in the l izards Anolis carolinensis (Jones and Guillette, 1982) and Hemidactylus flaviviridis (Prasad and Sanyal, 1969). Plasma T concentrations are significantly elevated above basal concentrations in the periovulatory period in T. s. parietalis (Whittier et al., 1987), N. naja (Bona-Gallo et al., 1980) and in the lizard Uromastix hardwicki (Arslan et al., 1978). Mean plasma T also rises during the mating period in the turtles Lepidochelys kempi (Rostal et al., 1998) and Geochelone nigra (Schramm et al., 1999), the tuatara Sphenodon punctatus (Cree et al., 1992), the viviparous snake Vipera aspis (Saint Girons et al., 1993) and occurs coincident with late vitellogenesis and the courtship/mating period in

female alligators, Alligator mississippiensis (Guillette et al., 1997). In addition, T is known to work synergistically with P4 to inhibit E2-induced vitellogenesis in reptiles (Ho et al., 1982; Ho, 1987), such that increased plasma T concentrations may cause the cessation of vitellogenesis immediately prior to ovulation (Ho et al., 1981) However, plasma T concentrations are not routinely measured in the examination of the female reproductive cycle in reptiles and the role of T is yet to be fully understood.

The present study examined the reproductive cycle of females of the viviparous lizard, Tiliqua nigrolutea. Individual females of this species do not produce a clutch every year (Edwards, 1999) allowing simultaneous comparison between reproductively active and quiescent females throughout the reproductive cycle. The timing of reproductive events was correlated with changes in mean plasma concentrations of P4, estrogen (E) and T throughout the annual cycle.

MATERIALS AND METHODS

Animals

Lizards were captured opportunistically by hand throughout southeastern Tasmania, Australia. Animals were housed at the University of Tasmania, Hobart (42°53'S, 147°19'E), in roofed outdoor enclosures 1.9 x 3.4 x 2.1 m; these were wire-fronted, allowing access to UV light and a natural photoperiod. The direct sunlight and a 120 W floodlight globe as an additional heat source at the front of each cage provided a temperature gradient across which the lizards could thermoregulate during their active season of the austral spring to mid-autumn (Sept - Apr). Bark and leaf litter were provided, in which the animals could hide. Mixed-sex groups of approximately five animals (one male per group) were maintained in these cages. Lizards were

maintained on a varied diet of fresh fruits, live snails and tinned catfood, provided two to three times weekly. Water was available ad libitum. Animals were observed hourly each day during the potential mating period (Oct – Nov) and when parturition was expected (Mar – Apr).

Blood sampling

A captive population of female T. nigrolutea was used for this study. All females were sampled as described; data for analyses were selected from reproductively active (N = 8) and quiescent (N = 8) females for which full data sets were obtained. The lizards were brought into the laboratory prior to sampling, and weight and snout-vent length (SVL) recorded. Blood samples were taken routinely between 0930 and 1230. Blood was collected from the caudal artery (without anaesthesia) using a heparinised syringe, and held on ice until centrifuging at 6400 rpm. Plasma was stored frozen at -20 °C until analysis. Up to 1 ml of blood was taken from each animal. During vitellogenesis, mating and ovulation, blood samples were collected fortnightly from all females, at the start of the first (A) and third (B) weeks of each month (Sept 1997 - Dec 1997). Monthly sampling of all individuals continued until the end of the active season (Jan 1998 - Apr 1998): animals hibernated from May – Aug. An additional blood sample was collected from each reproductively active female within 24 hr of parturition, and clutch number recorded. All hormone concentrations reported are means ± one standard error (1 SE).

Radioimmunoassays

Analytical reagent grade isooctane, hexane and ethanol were purchased from Biolab Scientific Pty. Ltd. (Victoria, Aust.). Scintillation fluid (Ecolite +) came from ICN (Costa Mesa, CA.). [1,2,6,7-^{3}H]-Testosterone (spec. act. 2.6-3.9 TBq mmol^{-1}) and [1,2,6,7-^{3}H]-P4 (spec. act. 3.0-4.1

TBq $mmol^{-1}$) were purchased from Amersham Life Sciences (UK). Testosterone antiserum was a gift from A. J. Bradley (details in Bradley, 1990). Plasma T concentrations were assayed by a modification to the radioimmunoassay of Castro et al. (1974) which has been published elsewhere (Swain and Jones, 1994). Intra- and interassay coefficients of variation for the testosterone assay were 6% and < 10%, respectively (Swain and Jones, 1994). Progesterone antiserum was from J. Malecki (details published in McDonald et al., 1988). The P4 radioimmunoassay method was described in Jones and Rose (1992) with a minor modification for this study: P4 was eluted from the columns in 3 ml isooctane. Intra- and interassay coefficients of variation for the P4 assay were 8.4% and 12.1% respectively. All T and P4 assay samples were measured as outlined in Jones and Rose (1992). Plasma E was measured using Spectria coated-tube radioimmunoassay kits as in Jones and Swain (1996). Cross-reactivities for the E2 antiserum are: E2, 100%; estrone (E1), 1.16%; estriol (E3), 0.45%; T and P4, <0.001%. Intra- and interassay coefficients of variation for this assay were 8% and 13%, respectively. The limit of detection for all three assays was 10 pg authentic steroid. Assays were validated using T. nigrolutea plasma (T and E assays) or pooled skink plasma (P4 assay): in all cases serial dilutions of plasma ran parallel to the standard curves.

Statistics

All statistical analyses were performed using SYSTAT 5.2 for the Macintosh (Wilkinson et al, 1992). A significance level of $\alpha = 0.05$ was used throughout. All data points were initially log-transformed to satisfy the assumptions of normality and homogeneity of variance. Occasional missing individual data were assigned the mean value for animals of like reproductive status in the same sample period, although no more than one such value was assigned to any sample set or any individual animal (Mundry, 1999; D. Ratkowsky, pers. comm.). Data from the initial

sampling period (Sept A) was not included in analyses as some animals had not yet emerged from hibernation at that time. Annual patterns of mean plasma steroid concentrations were examined in both pregnant and quiescent female T. nigrolutea by two-way repeated measures Analysis of Variance ((M)ANOVA). The multivariate output of these analyses (Pillai trace statistic) tested both for changes in mean plasma steroid concentration through time and for time-state interactions, where "state" was the reproductively active or quiescent condition of the lizards. Multivariate analysis was used because the univariate output from the SYSTAT programme is unsuitable due to a lack of independence of the data through time (C. Johnson, pers. comm.). The precise timing of ovulation and the mating period was unknown before blood samples were collected; therefore, a posteriori unpaired Student t tests were conducted to compare mean values for pregnant and quiescent animals for each steroid hormone profile (C. Johnson, pers. comm.). Visual examination of completed hormone profiles and behavioral observations of the captive population concurrent with blood sampling were used to identify relevant successive pairs of sample sets.

RESULTS

Progesterone

A seasonal pattern of variations in plasma P4 concentrations was evident. A comparison of mean plasma P4 concentrations in reproductively active and quiescent female T. nigrolutea throughout the reproductive season (spring (Sept) 1997 - autumn (Apr) 1998) is shown in Figure 1. In quiescent females, mean plasma P4 concentration was low (1.1 $\pm$ 0.20 ng ml^{-1}) at emergence in early spring (Sept B) and remained basal (1 - 2 ng ml^{-1}) throughout the active season. In reproductively active females, mean plasma P4 concentration became significantly elevated in

mid spring (Nov A), corresponding to the late vitellogenic/early mating and ovulatory period ((M)ANOVA: $F_{(7,7)} = 52.600$, $\underline{P} = 0.000$) and remained high, peaking in the second trimester (Jan) of gestation (12.7 ± 1.27 ng ml^{-1}). Mean plasma P4 concentrations fell (4.4 ± 0.88 ng ml^{-1}) by late summer (Feb), but prior to parturition and returned to basal concentrations by early autumn, during the parturition period (Mar – Apr).

Multivariate analysis also revealed a significant interaction effect between time and condition of females (reproductive or quiescent) ((M)ANOVA: $F_{(1,7)} = 11.634$, $\underline{P} = 0.007$). A paired Student t test revealed no significant change in mean plasma P4 concentration between late pregnancy (Feb) and within 24 hr of parturition. There was no correlation between peak (mid summer (Jan)) mean plasma P4 concentration of reproductively active females and female snout-vent length (SVL), the number of offspring produced, or the number of CLs present (extrapolated from the number of offspring plus unfertilised ova produced).

Estrogen

Mean plasma E concentration varied significantly throughout the active season ((M)ANOVA: $F_{(1,15)} = 354.117$, $\underline{P} = 0.000$) (Figure 2). There was a significant interaction effect between time of year and reproductive condition of females ((M)ANOVA: $F_{(1,7)} = 5.903$, $\underline{P} = 0.032$). While mean plasma E concentrations in quiescent females did fluctuate throughout the active season, there were no significant changes with time. In contrast, mean plasma E concentration in reproductively active females was low (275.2 ± 36.87 pg ml^{-1}) at spring emergence (early spring (Sept B)) and increased during the mid spring vitellogenic period (Oct A and B), peaking later in spring, immediately prior to ovulation (Nov A) at 715.1 ± 106.68 pg ml^{-1}. A posteriori unpaired Student t

tests demonstrated that mean plasma E concentrations in reproductively active females were elevated significantly above those of quiescent females at times corresponding to late vitellogenesis (mid spring (Oct B)) (t test: t = -2.337, df = 14, P = 0.035) and mating/ovulation (late spring (Nov A)) (t test: t = -3.032, df = 14, P = 0.009). A paired Student t test showed no significant change in mean plasma E concentration between late gestation (Feb) and within 24 hr of parturition, and there was no correlation between peak (late spring (Nov A)) mean plasma E concentration of reproductively active females and their SVL or the number of offspring produced.

Testosterone

Significant variations in mean plasma T concentrations in reproductive females were observed through the active season ((M)ANOVA: $F_{(1, 7)} = 46.339$, P = 0.000) and a similar pattern was observed in quiescent females (Figure 3). The cycle was characterised by a peak in plasma T concentrations in late spring, during the mating/ovulation period (Nov B) (reproductive: 6.3 ± 0.63 ng ml^{-1}, quiescent: 4.7 ± 0.31 ng ml^{-1}) and a marked decline to < 1 ng ml^{-1} by early summer (the start of gestation in pregnant lizards) (Dec A) in all individuals. An a posteriori unpaired t test showed a significant elevation in mean plasma T concentration in reproductively active females above that of quiescent individuals in late spring (Nov B) (t test: t = -2.415, df = 14, P = 0.030); this corresponded to the mating and ovulatory period in reproductively active females. There was no significant change (paired Student t test) in mean plasma T concentration between late gestation and within 24 hr of parturition, and there was no correlation between peak (late spring (Nov B)) mean plasma T concentration of reproductively active females and their SVL or the number of offspring produced.

DISCUSSION

The changes in mean plasma P4 concentration described for female T. nigrolutea throughout the active season are typical of those observed in many other temperate zone viviparous squamates (Chan et al., 1973; Bourne et al., 1986; Kleis-San Francisco and Callard, 1986; Fergusson and Bradshaw, 1991; Van Wyk, 1994; Jones and Swain, 1996; Jones et al., 1997). The magnitude of peak mean plasma P4 concentration shows little variation between species with similar plasma P4 profiles (Cordylus giganteus: 5 ng ml^{-1} (Van Wyk, 1994), Niveoscincus ocellatus: 6.5 ng ml^{-1} (Jones et al., 1997), Nerodia sp.: 10 ng ml^{-1} (Kleis-San Francisco and Callard, 1986), Niveoscincus metallicus: 11.5 ng ml^{-1} (Jones and Swain, 1996) and all are comparable with T. nigrolutea (12.7 ± 1.27 ng ml^{-1}).

The specific role of P4 in gestation remains unclear. Progesterone may function to slow the rate of ovarian development, delaying the next ovulation and gestation, so that young are born into conditions optimal for their survival (Callard et al., 1992); it may influence the rate of development (Gemmell, 1995), or delay parturition by reducing oviductal contractility (Guillette et al., 1991). It has been proposed that the evolution of viviparity as a response to cold climates was facilitated by the effects of P4 on uterine motility (Guillette et al., 1981; Guillette and Jones, 1985), gestation length (Callard et al., 1972a, b; Highfill and Mead, 1975b) and oviductal hypertrophy (Guillette and Jones, 1985), resulting in more prolonged gestation (Callard et al., 1972a, b; Shine and Guillette, 1988). Elevated plasma P4 concentrations during gestation also inhibit E2-stimulated follicular growth (Yaron and Widzer, 1978), so that vitellogenesis is not initiated during pregnancy (Callard et al., 1992). This effect of P4 on follicular growth is termed

"antigonadal" (Callard et al., 1972a; Guillette et al., 1981; Ho, 1987) and is important for temperate zone viviparous reptiles which are usually constrained by a limited active season to a single reproductive effort each year (Shine, 1985; Dunham et al., 1988). The decline in mean plasma P4 seen well before parturition in T. nigrolutea is likely to be related to the antigonadal actions of this hormone, and to reflect the multihormone control of gestation and parturition.

In reproductively active female T. nigrolutea, annual changes in mean plasma E concentrations reflect important physiological events in the reproductive cycle, rising during vitellogenesis and ovulation. A similar plasma E profile is displayed in many other viviparous squamates (Callard et al., 1972c; Kleis-San Francisco and Callard, 1986; Callard and Kleis, 1987; Bonnet et al., 1994; Van Wyk, 1994; Jones and Swain, 1996; Jones et al., 1997), although there is considerable variation in the magnitude of peak mean plasma E2 concentr ations between species. Among viviparous squamates, peak E concentrations at ovulation range from 700 -1300 pg ml^{-1} in T. nigrolutea (this study), N. metallicus (Jones and Swain, 1996), N. ocellatus (Jones et al., 1997) and Nerodia sp. (Kleis-San Francisco and Callard, 1986), to 4 ng ml^{-1} in V. aspis (Saint Girons et al., 1993; Bonnet et al., 1994), but rarely as high as concentrations seen in C. giganteus, in which plasma E2 peaks above 600 ng ml^{-1} (Van Wyk, 1994). However, regardless of the differences in the magnitude of plasma E peaks between species, the patterns of change throughout the reproductive cycle remain the same. In reproductively active female T. nigrolutea mean plasma E rises again at parturition, although it does not become significantly ele vated above concentrations detected in quiescent females. A similar trend has been reported in two other viviparous squamates, the lizard C. giganteus (Van Wyk, 1994) and the snake Nerodia sp. (Kleis-San Francisco and Callard, 1986).

Although only examined in females of a few, usually oviparous, reptile species, T appears to be an important secretory product of the ovary (Arslan et al., 1978; Callard et al., 1978; Owens, 1997; Staub and De Beer, 1997). Both reproductively active and quiescent female T. nigrolutea display variation in mean plasma T concentrations throughout the active season. The changes observed in reproductively active females are also apparent, but at lower concentrations, in quiescent individuals. This phenomenon has also been reported in the viviparous snake T. s. parietalis (Whittier et al., 1987). In reproductively active female T. nigrolutea mean plasma T increases through late vitellogenesis, peaking at 6.3 ± 0.63 ng ml $^{-1}$ at ovulation and then declining rapidly. The magnitude of this T peak is relatively high for a female reptile. By comparison, plasma T in females peaks between 300 and 400 pg ml $^{-1}$ in the oviparous snake N. naja (Bona-Gallo et al., 1980) and the turtles Caretta caretta (Wibbels et al., 1990) and L. kempi (Rostal *et al*., 1998). Peak plasma T is higher in female Alligator mississippiensis (1.12 ng ml^{-1}) (Guillette et al., 1997), T. s. parietalis (2 ng ml^{-1}) (Whittier et al., 1987) and the turtle Chrysemys picta (4.5 ng ml^{-1}) (Callard et al., 1978). Only the tuatara S. punctatus (11.4 ng ml^{-1}) (Cree et al., 1992) is reported to have a higher peak plasma concentration of T in females than T. nigrolutea. Such differences may reflect species-specific variation in the specificity or capacity of sex steroid binding proteins (Paolucci et al., 1992).

The pattern of seasonal changes in mean plasma T concentrations in T. nigrolutea is very similar to that seen in some other female reptiles (Bona-Gallo et al., 1980; Callard and Kleis, 1987; Whittier et al., 1987; Saint Girons et al., 1993). Although the majority of published studies have examined non-squamate species, a correlation between elevated plasma T concentrations and

vitellogenesis and ovulation or mating is reported (Callard et al., 1978; Moore, 1986; Wibbels et al., 1990; Cree et al., 1992; Guillette et al., 1997; Rostal et al., 1998). Testosterone is also known to stimulate oviductal hypertrophy (Jones and Guillette, 1982) and uterine development in ovariectomised lizards (Yaron, 1972b). However, few studies of plasma T concentrations in female viviparous squamates are available for comparison.

The presence of T at physiologically relevant concentrations in plasma implies a biological function for this hormone beyond its role as a precursor for the synthesis of estrogen (Staub and De Beer, 1997). The profile of mean plasma testosterone in female T. nigrolutea provides circumstantial evidence that T may be involved in the regulation of vitellogenesis and ovulation in this species. However, while a temporal correlation between elevated plasma T and vitellogenesis and ovulation has been demonstrated in a number of female reptiles, a causal relationship is yet to be proven for any species.

In addition to their individual actions, the combined effects of gonadal steroids in the regulation of reproductive physiology must be considered. Plasma profiles of P4, E and T in reproductively active female T. nigrolutea suggest that the key reproductive events of vitellogenesis, ovulation, gestation and parturition are under multihormone control. To facilitate discussion, the seasonal profiles of plasma P4, E and T in female T. nigrolutea are shown overlaid in Figure 4.

In T. nigrolutea, mean plasma E, T and P4 concentrations rise during the late vitellogenic period. In other reptiles, E2 and T are synthesised by growing follicles: plasma concentrations increase as follicles enlarge (Wade 1997; Staub and De Beer, 1997) and then fall in the periovulatory period

(Callard and Kleis, 1987; Wibbels et al., 1990; Guillette et al., 1997; Rostal et al., 1998). Estrogens and progestins secreted by the ovary in the pre-ovulatory period act synergistically to stimulate maturation of the genital tract in female reptiles (Yaron, 1972a). The release of the ovum is thought to deactivate the enzyme systems responsible for the further conversion of P4 to E2 (Yaron, 1972a). Correspondingly, at ovulation in T. nigrolutea, plasma E and T concentrations decrease and mean plasma P4 concentration rises. Progesterone and T have marked antagonistic effects on E2-induced vitellogenin synthesis in reptiles (Giannoukos and Callard, 1995) and may function to terminate vitellogenesis after ovulation (Ho et al., 1982; Callard et al., 1992). This is accomplished at two levels; the liver is the site of one such antigonadal effect, where T regulates the stimulatory action of E2 (Callard and Ho, 1987). Simultaneously, at the follicular level P4 inhibits the incorporation of vitellogenin into oocytes (Yaron and Widzer, 1978). In T. nigrolutea, mating occurs immediately prior to ovulation, coincident with peak plasma E, and a small peak in plasma P4.

During gestation in T. nigrolutea, elevated plasma P4 and T (presumably of ovarian origin) may inhibit E production and prevent further follicular development, as described in other reptiles (Callard et al., 1972a; Ho et al., 1982; Callard et al., 1992). Alternatively, inhibition may be accomplished at the receptor level; P4 causes downregulation of E2 receptors in the brain of the lizards Cnemidophorus inornatus and Cnemidophorus uniparens (Godwin et al., 1996). The rise in plasma E concentration seen near the end of gestation in T. nigrolutea has been associated with the onset of parturition in the related species T. rugosa (Fergusson and Bradshaw, 1992) and occurs coincident with falling P4 and low T concentrations in the plasma.

Although experimental evidence is still required to support this hypothesis, the plasma steroid profiles of P4, E and T in reproductively active female T. nigrolutea (Figure 4) suggest the multihormonal control of reproduction in females of this species. This highlights the importance of considering, not only the direct actions of reproductive steroid hormones, but also their potential interaction with other steroids and the regulation of receptor numbe rs, in the interpretation of results from castration or hormone manipulation studies, or when characterising reptilian reproductive cycles.

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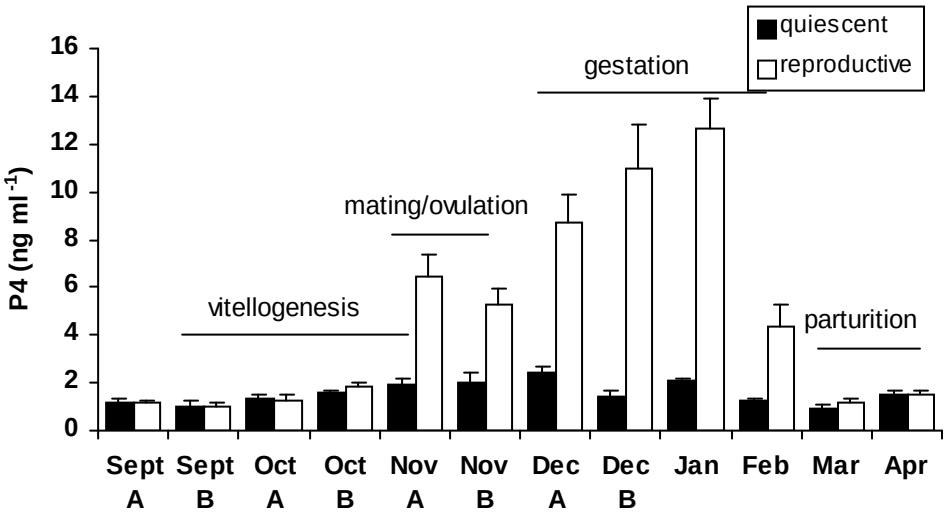
Figure Legends

Figure 1. Plasma P4 concentrations in reproductively active (N = 8) and quiescent (N = 8) female Tiliqua nigrolutea throughout the active season. Sampling was fortnightly from September 1997 to December 1997 (samples designated A and B within these months) and then monthly until April 1998. Reproductive conditions corresponding to monthly samples were: vitellogenesis (Sept A – Nov A); ovulation and mating (Nov A – Nov B); gestation (Dec A – Feb); parturition (Mar – Apr). Values are means ± 1 standard error.

Figure 2. Plasma E2 concentrations in reproductively active (N = 8) and quiescent (N = 8) female Tiliqua nigrolutea throughout the active season. Sampling was fortnight ly from September 1997 to December 1997 (samples designated A and B within these months) and then monthly until April 1998. Reproductive conditions corresponding to monthly samples were: vitellogenesis (Sept A – Nov A); ovulation and mating (Nov A – Nov B); gestation (Dec A – Feb); parturition (Mar – Apr). Values are means ± 1 standard error.

Figure 3. Plasma T concentrations in reproductively active (N = 8) and quiescent (N = 8) female Tiliqua nigrolutea throughout the active season. Sampling was fortnig htly from September 1997 to December 1997 (samples designated A and B within these months) and then monthly until April 1998. Reproductive conditions corresponding to monthly samples were: vitellogenesis (Sept A – Nov A); ovulation and mating (Nov A – Nov B); gestation (Dec A – Feb); parturition (Mar – Apr). Values are means ± 1 standard error.

Figure 4. Mean plasma P4, E and T concentrations in reproductively active female Tiliqua nigrolutea (N = 8) throughout the active season, overlaid to demonstrate t he potential timing of in vivo steroid hormone interactions. Sampling was fortnightly from September 1997 to December 1997 (samples designated A and B within these months) and then monthly until April 1998. Reproductive conditions corresponding to monthly samples were: vitellogenesis (Sept A – Nov A); ovulation and mating (Nov A – Dec A); gestation (Dec A – Feb); parturition (Mar – Apr). Standard errors are omitted for clarity.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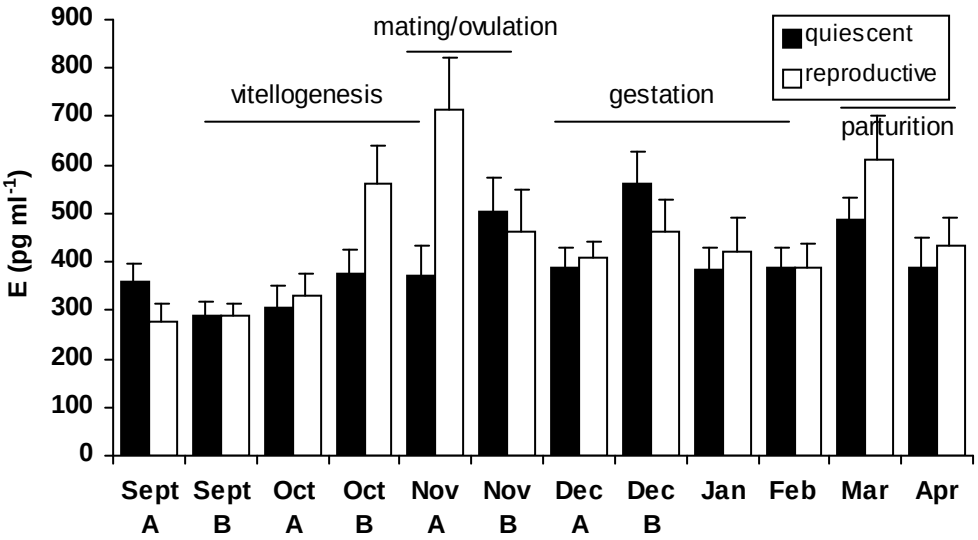
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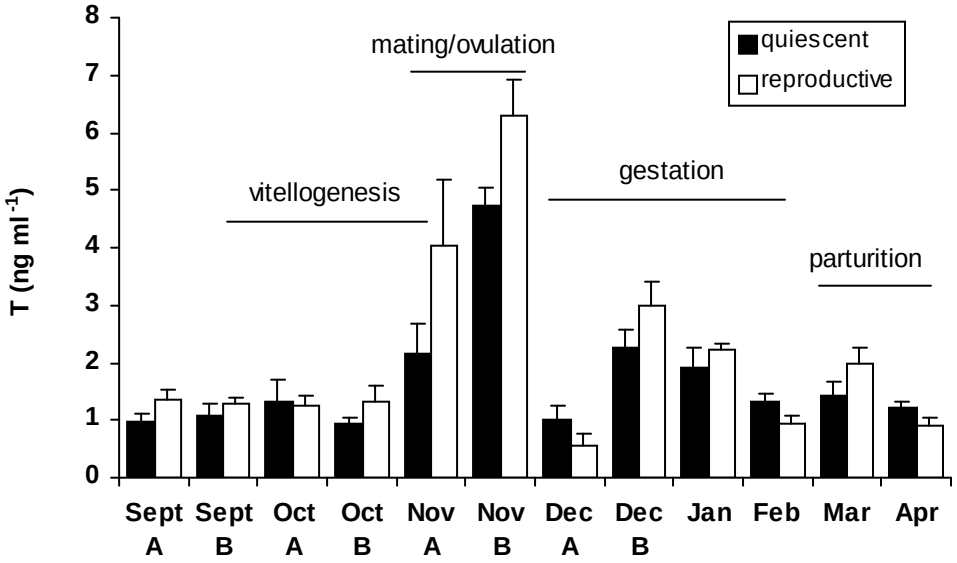
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mating/ovulation
vitellogenesis
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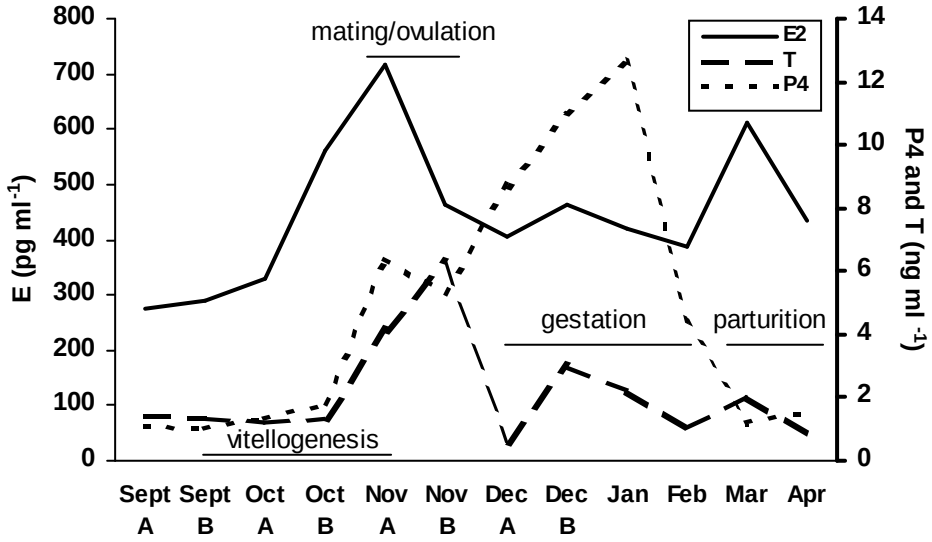
mating/ovulation
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800
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